

Research Note

Surgical Implantation of *Echinostoma caproni* Metacercariae and Adults into the Small Intestine of ICR Mice

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ABSTRACT: Encysted and excysted metacercariae or adults of *Echinostoma caproni* were surgically implanted into the small intestine of ICR mice. Worm recovery at 10 days postimplantation from mice that had received either 25 encysted or 25 excysted metacercariae was 93%. Worm recovery at 7 days postimplantation from mice that had previously received 25 7-day-old worms was 64%. Worms recovered from surgically implanted hosts showed similar growth and development patterns to those obtained from hosts fed cysts by stomach tube.

KEY WORDS: *Echinostoma caproni*, ICR mouse, metacercariae, surgical implantation.

Recent studies have demonstrated that laboratory mice are excellent experimental hosts for the intestinal trematode, *Echinostoma caproni*, with infection rates of 100% and worm recoveries of 45–95% (Odaibo et al., 1988; Hosier and Fried, 1991). These studies used 25 encysted metacercariae per mouse, and cysts were administered by stomach tube. Less information is available on the surgical implantation of echinostome metacercariae or adults into the small intestine of mice. Christensen et al. (1986) surgically implanted excysted metacercariae of *E. caproni* (referred to as *E. revolutum* in that paper) into the mouse duodenum to examine host immunogenic effects on that larval stage. Nollen (1990) surgically transplanted radioisotopically labeled adults of *E. caproni* into the mouse small intestine to study the reproductive physiology of these worms. We have not found reference to studies on the surgical implantation of echinostome cysts into the mouse gut. The present study was undertaken to determine the efficacy of implanting encysted and excysted metacercariae, and young adults of *E. caproni*, into the small intestine of ICR mice.

Encysted metacercariae were removed from experimentally infected *Biomphalaria glabrata* snails and fed by stomach tube (25 cysts/host) or implanted surgically (25 cysts/host) into the mouse small intestine as described below. Excysted metacercariae were obtained following

metacercarial excystation in an alkaline trypsin-bile salts solution (Fried and Emili, 1988) and implanted surgically. Adults, 7 days old, were removed from donor hosts and implanted surgically (25/host) into the mouse duodenum as follows.

Mice were fasted for 12 hr before surgery. Under sodium pentobarbital anesthesia (60 mg/kg body weight), a 10–15-mm incision was made in the upper abdomen. The stomach and first 20–25 mm of the small intestine were pulled through the incision, and a 3–5-mm slit was made in the stomach wall. *Echinostoma caproni* adults or metacercariae were drawn into polyethylene tubing (0.86 mm inner diameter) attached to a 1-ml tuberculin syringe, and the tubing was inserted into the slit and gently manipulated past the pyloric valve into the first 10–15 mm of the duodenum. Adults or metacercariae were injected with 0.1–0.2 ml Locke's solution. The incisions were closed with 5/0 sutures.

At necropsy, mice were anesthetized lightly with ether and killed by cervical dislocation, and the small intestine was examined for worms. Mice receiving larvae either orally or surgically were necropsied 10 days postinfection. Mice receiving adults were necropsied 7 days postimplantation. Worms recovered from mice were in the lower part of the small intestine, usually 20–40 cm from the pylorus. They were fixed in hot alcohol-formalin-acetic acid, stained in carmine and mounted in Permount, and body area measurements were made (Hosier and Fried, 1991).

The results of the experiments are summarized in Table 1. There was no difference in worm recovery or worm body area among mice that received encysted metacercariae by stomach tube (group A), encysted metacercariae by surgery (group B), or excysted metacercariae by surgery (group C, ANOVA, $P > 0.05$). Of the 20 fixed and stained worms from groups A, B, and C, 19 were ovigerous and 1 from group B was previgorous. The infectivity, growth, and development

Table 1. Summary of transplantation experiments with *Echinostoma caproni* larvae and adults in ICR mice.

Group*	No. of infected mice†	Mean number \pm SE of worms recovered per host‡	No. of worms measured	Mean \pm SE of body area (mm ²) of worms
A	5	20.4 \pm 1.7	10	2.3 \pm 0.19
B	5	22.6 \pm 1.3	5	1.70 \pm 0.17
C	5	22.8 \pm 1.7	5	2.02 \pm 0.21
D	2	16	6	3.10 \pm 0.26

* All mice received 25 worms as follows: A, cysts fed by stomach tube; B, cysts implanted surgically; C, excysted metacercariae implanted surgically; and D, 7-day-old adults implanted surgically.

† All mice exposed to larvae or adults became infected.

‡ Worms were recovered 10 days postinfection except in D, where recoveries were made at 7 days postimplantation.

studies showed that surgical implantation of either encysted or excysted metacercariae is feasible for studies on *E. caproni* in ICR mice. Encysted metacercariae of this species can excyst in vivo without passage through the stomach. Fried and Emili (1988) reported that encysted metacercariae of this species can be excysted in vitro in the absence of acid-pepsin pretreatment. Surgical implantation of 7-day-old adults into the mouse gut resulted in a worm recovery rate of 64% at 7 days postimplantation. These 14-day-old worms (7 days in donor plus 7 days in recipient) were ovigerous with a mean body area

of 3.1 mm² (see D, Table 1). Ten worms grown for 7 days in mice were preovigerous with a mean body area of 0.85 \pm 0.07 mm². Data from Hosier and Fried (1991) showed that 30 worms grown for 14 days in mice were ovigerous with a mean body area of 5.2 \pm 0.4 mm². The difference in body area (Student's *t*-test, *P* < 0.05) of the 14-day-old worms grown in our study compared to that seen in Hosier and Fried (1991) may reflect some retardation of growth in worms transplanted to a new host. The surgical implantation procedure described herein is suitable for echinostome adults as well as metacercariae.

Literature Cited

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